Lung transplantation with cardiopulmonary bypass exaggerates pulmonary vasomotor dysfunction in the transplanted lung

Pulmonary vascular resistance is significantly increased in the transplanted lung. If cardiopulmonary bypass is required, the transplanted lung is reperfused with activated blood elements, which might exacerbate the reperfusion injury. The purpose of this study was to examine the influence of cardiopulmonary bypass on the following mechanisms of pulmonary vasomotor control in a dog model of autologous lung transplantation: (1) endothelium-dependent cyclic guanosine monophosphate–mediated relaxation (response to acetylcholine), (2) endothelium-independent cyclic guanosine monophosphate–mediated relaxation (response to nitroprusside), and (3) β-adrenergic cyclic adenosine monophosphate–mediated relaxation (response to isoproterenol). Autologous right lung transplants were performed with (n = 4 dogs) and without (n = 5 dogs) bypass. Lungs were stored in cold saline solution (4° C, 3 hours) before reimplantation. Pulmonary vasomotor control mechanisms were studied in isolated pulmonary arterial rings immediately after harvest and 1 hour after reimplantation. Ten rings were studied in each group at each time. Statistical analysis was by analysis of variance. Without bypass, endothelium-dependent cyclic guanosine monophosphate–mediated relaxation and β-adrenergic cyclic adenosine monophosphate–mediated relaxation were significantly impaired, although endothelium-independent cyclic guanosine monophosphate–mediated relaxation was not. Use of bypass produced significantly greater impairment of both endothelium-dependent cyclic guanosine monophosphate–mediated relaxation and β-adrenergic cyclic adenosine monophosphate–mediated relaxation. In addition, use of bypass produced significant dysfunction of endothelium-independent cyclic guanosine monophosphate–mediated relaxation as well. We conclude that using cardiopulmonary bypass to perform lung transplantation greatly exaggerates pulmonary vasomotor dysfunction in the transplanted lung. This dysfunction may contribute to significantly higher pulmonary vascular resistance in the transplanted lung if cardiopulmonary bypass is used. (J THORAC CARDIOVASC SURG 1995;109:212-7)

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Lung transplantation requires the transplanted lung to endure the injuries of both cold ischemia and reperfusion. Together, these produce pulmonary vasomotor dysfunction in the transplanted lung.[1] These injuries are associated with an acute rise in pulmonary vascular resistance (PVR) in the transplanted lung.[2-12] This increased PVR is largely due to avid constriction of the pulmonary vascular smooth muscle, even in the absence of hypoxia.[10, 12] PVR may remain chronically elevated in laboratory models of lung transplantation and is not attributable to rejection; PVR remains elevated even in models of canine autologous lung transplantation.[9, 12]

If cardiopulmonary bypass is necessary for lung transplantation, the transplanted lung is reperfused with activated blood elements that may exacerbate

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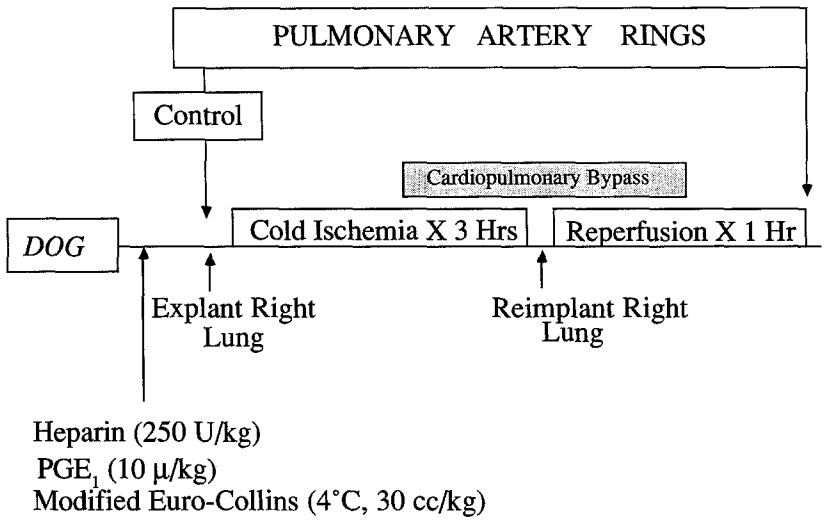

Fig. 1. Experimental protocol. Pulmonary arterial rings were examined immediately after lung explanation (control) and 1 hour after lung reimplantation. In the group in which cardiopulmonary bypass was used, bypass was begun 30 minutes before lung reimplantation. The dogs were weaned from bypass 30 minutes after reimplantation. *PGE_1*, Prostaglandin E_1.

the reperfusion injury. We hypothesized that pulmonary vasomotor dysfunction is exaggerated when cardiopulmonary bypass is used for lung transplantation. The purpose of this study was to examine the influence of cardiopulmonary bypass on the following pulmonary vasomotor control mechanisms in a canine model of autologous lung transplantation: (1) endothelium-dependent cyclic guanosine monophosphate (cGMP)–mediated relaxation (response to acetylcholine), (2) endothelium-independent cGMP-mediated relaxation (response to sodium nitroprusside), and (3) β-adrenergic cyclic adenosine monophosphate (cAMP)–mediated relaxation (response to isoproterenol). This study demonstrates that the use of cardiopulmonary bypass to perform lung transplantation greatly exaggerates dysfunction of each of these mechanisms of pulmonary vasomotor control in the transplanted lung.

Methods

Surgical protocol. All animals received humane care in compliance with the "Principles of Laboratory Animal Care" formulated by the National Society for Medical Research and the "Guide for the Care and Use of Laboratory Animals" prepared by the Institute of Laboratory Animal Resources and published by the National Institutes of Health (NIH Publication No. 86-23, revised 1985).

Right autologous lung transplantation was performed through a right thoracotomy incision in mongrel dogs (25 to 35 kg) after endotracheal intubation and mechanical ventilation with pentobarbital and halothane anesthesia. Blood pressure was continuously monitored with an intraarterial catheter. One group of five dogs underwent lung transplantation without cardiopulmonary bypass. In another group of four dogs the right lung was explanted and cardiopulmonary bypass was subsequently initiated 30 minutes before lung reimplantation. In the latter group, cardiopulmonary bypass was performed with bicaval cannulation and a bubble oxygenator (Cobe Laboratories, Denver, Colo.). The experimental protocol is shown in Fig. 1.

After systemic heparinization (250 units/kg), prostaglandin E_1 (10 μg/kg) was infused distally into the right main pulmonary artery. Then, with the right lung inflated and the pulmonary veins vented into the right side of the chest, the right main pulmonary artery was occluded proximally as modified Euro-Collins solution (4° C, 30 ml/kg) was infused distally into the right pulmonary artery. With the use of a balloon bronchial occluder, the right lung was explanted and maintained inflated by clamping the right main stem bronchus. It was stored in cold saline solution (4° C) for 3 hours. Thereafter, the right lung was surgically reimplanted with a running monofilament suture technique for the bronchial, the arterial, and the venous anastomoses. The pulmonary arterial clamp was removed and the lung was reperfused and ventilated for approximately 1 hour. In the group in which cardiopulmonary bypass was used, bypass was begun 30 minutes before lung reimplantation. The lung was reperfused and ventilated for 30 minutes during bypass. The animals were then weaned from bypass and the lung ventilated for an additional 30 minutes off bypass.

Pulmonary artery ring preparation. With the use of a dissecting microscope, third-order pulmonary arteries (approximately 1 mm in diameter) were dissected from each lung and studied at each of two times: (1) immediately after harvest (control) and (2) 1 hour after lung

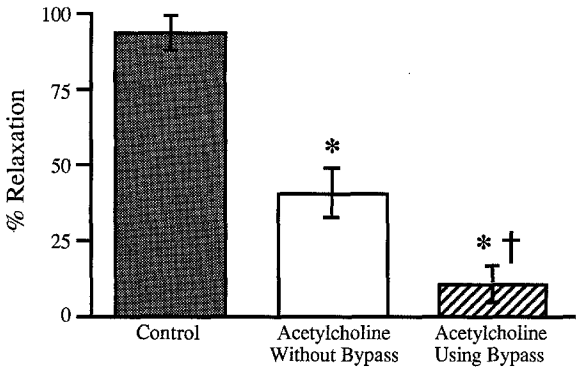

Fig. 2. Endothelium-dependent cGMP-mediated vasorelaxation. Relaxation by acetylcholine was significantly impaired in the transplanted lung without use of cardiopulmonary bypass. This dysfunction was significantly exaggerated by the use of cardiopulmonary bypass. $n = 10$ rings in each group. Values are mean ± standard error of the mean. $*p < 0.05$ compared with control. $\dagger p < 0.05$ versus without use of cardiopulmonary bypass.

reimplantation. Under dissecting microscope magnification, the surrounding tissue was dissected from the pulmonary arteries. The pulmonary arteries were then cut into rings, each ring being 3 to 4 mm wide. Great care was taken during this process to avoid endothelial injury. The pulmonary arterial rings were suspended on fine wire tensiometers in individual 10 ml organ chambers. The organ chambers were surrounded by water jackets and continually warmed to 37° C. Ring tension was determined by use of a force-displacement transducer (Grass FTO3, Grass Instruments Co., Quincy, Mass.) attached to each tensiometer apparatus. Ring tension was thereby recorded with a MacLab data interface module (ADI Instruments, Milford, Mass.) on a Macintosh IIci computer (Apple Computer, Inc, Cupertino, Calif.). The organ chambers were filled with Earle's balanced salt solution and continuously bubbled with gas consisting of 21% oxygen, 5% carbon dioxide, and balanced nitrogen. Earle's balanced salt solution is a standard physiologic salt solution and contains $CaCl_2$ 1.80 mmol/L, $MgSO_4$ (anhydrous) 0.83 mmol/L, KCl 5.36 mmol/L, NaCl 116.34 mmol/L, $NaPO_4$ 0.40 mmol/L (dibasic), D-glucose 5.50 mmol/L, $NaHCO_3$ 19.04 mmol/L, and phenol red Na 0.03 mmol/L (as pH indicator). The oxygen tension of the bath was 100 to 110 mm Hg and the pH was 7.35 to 7.45.

In a separate series of experiments, the concentration of acetylcholine, sodium nitroprusside, and isoproterenol required to produce complete relaxation of isolated canine pulmonary arterial rings was determined to be 10^{-6} mol/L for each agent. The rings in this study were allowed to reach a steady state (approximately 90 minutes) at a baseline tension of 750 mg. The rings were then preconstricted with phenylephrine 10^{-6} mol/L to study the vasorelaxing effects of acetylcholine 10^{-6} mol/L, sodium nitroprusside 10^{-6} mol/L, and isoproterenol 10^{-6} mol/L in each ring in a random order. Once ring tension reached a steady state in response to phenylephrine, a given vasorelaxing agent was added to the organ chamber. After each agent was tested, the organ chambers were flushed several times and the rings were allowed to reach a steady state before being preconstricted once again with phenylephrine to test the next vasorelaxing agent. Ten pulmonary arterial rings in each group were studied at the point of data collection. Data are presented as the percent relaxation of phenylephrine-induced ring tension produced by the given vasorelaxing agent. Values are expressed as mean plus or minus one standard error of the mean. Statistical analysis was done by analysis of variance (Scheffe's F test). A p value of less than 0.05 was considered statistically significant.

Results

Pulmonary vasomotor dysfunction was greatly exaggerated when cardiopulmonary bypass was used to perform lung transplantation. Immediately after lung harvest, acetylcholine 10^{-6} mol/L produced 95% ± 5% relaxation, sodium nitroprusside 10^{-6} mol/L produced 98% ± 2% relaxation, and isoproterenol 10^{-6} mol/L produced 92% ± 5% relaxation in control vessels. Without the use of cardiopulmonary bypass, endothelium-dependent cGMP-mediated relaxation was significantly impaired because acetylcholine produced only 46% ± 10% relaxation ($p < 0.05$ versus control). This dysfunction was significantly exaggerated by the use of cardiopulmonary bypass: acetylcholine produced only 8% ± 6% relaxation ($p < 0.05$ versus without bypass) (Fig. 2).

On the other hand, endothelium-independent cGMP-mediated relaxation was not impaired without the use of cardiopulmonary bypass: sodium nitroprusside produced 95% ± 4% relaxation. However, cardiopulmonary bypass resulted in significant dysfunction of this mechanism of pulmonary vasorelaxation because sodium nitroprusside produced only 43% ± 6% relaxation if bypass was used ($p < 0.05$ versus without bypass) (Fig. 3).

β-Adrenergic cAMP-mediated vasorelaxation was significantly impaired even without the use of cardiopulmonary bypass: isoproterenol produced 38% ± 6% relaxation ($p < 0.05$ versus control). This dysfunction was also significantly exaggerated by the use of cardiopulmonary bypass. If bypass was used, isoproterenol produced only 22% ± 5% relaxation ($p < 0.05$ versus without bypass) (Fig. 4).

Discussion

The ischemic and reperfusion injuries incurred by the transplanted lung produce significant dysfunction of the mechanisms of pulmonary vasorelaxation.[1] The results of the present study demonstrate that this vasomotor dysfunction is significantly exag-

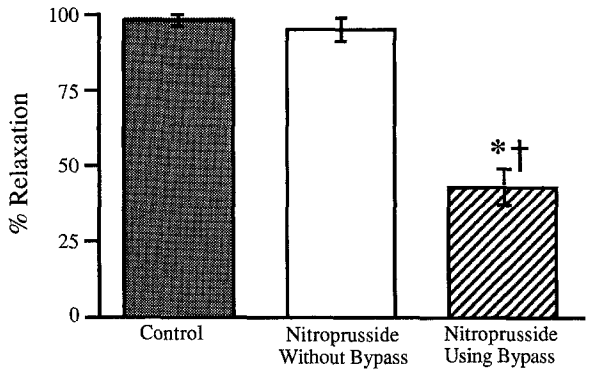

Fig. 3. Endothelium-independent cGMP-mediated vasorelaxation. Relaxation by sodium nitroprusside was not impaired in the transplanted lung without use of cardiopulmonary bypass. However, use of cardiopulmonary bypass resulted in significant dysfunction. $n = 10$ rings in each group. Values are mean ± standard error of the mean. $*p < 0.05$ compared with control. $\dagger p < 0.05$ versus without use of cardiopulmonary bypass.

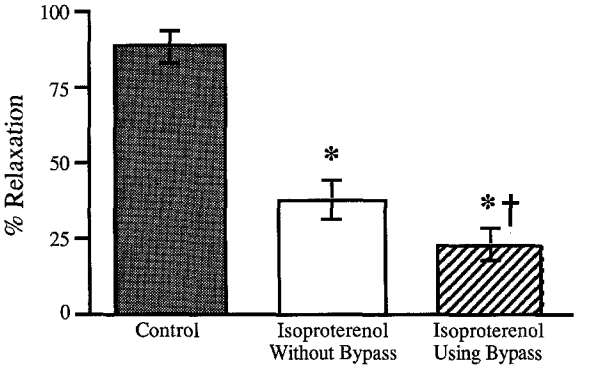

Fig. 4. β-Adrenergic cAMP-mediated vasorelaxation. Relaxation by isoproterenol was significantly impaired in the transplanted lung without use of cardiopulmonary bypass. This dysfunction was significantly exaggerated by the use of cardiopulmonary bypass. $n = 10$ rings in each group. Values are mean ± standard error of the mean. $*p < 0.05$ compared with control. $\dagger p < 0.05$ versus without use of cardiopulmonary bypass.

gerated if cardiopulmonary bypass is used to perform the lung transplant operation.

The principal intracellular mechanisms of pulmonary vascular smooth muscle relaxation are ultimately mediated through adenosine 3′,5′-cyclic monophosphate (cAMP) or guanosine 3′,5′-cyclic monophosphate (cGMP).[13] In response to activation of receptors on pulmonary vascular smooth muscle cells (such as β-adrenergic receptors), pulmonary vascular smooth muscle adenylate cyclase generates cAMP, which in turn effects pulmonary vasorelaxation.[14] On the other hand, cGMP-mediated relaxation may be either endothelium dependent or endothelium independent. Agents such as acetylcholine produce pulmonary vascular smooth muscle relaxation by binding to muscarinic receptors on pulmonary vascular endothelium. In response, the pulmonary vascular endothelium releases endothelium-derived relaxing factor, which is thought to be nitric oxide. Endothelium-derived relaxing factor in turn activates guanylate cyclase within the pulmonary vascular smooth muscle cell.[13] The cGMP thereby produced via guanylate cyclase effects pulmonary vascular smooth muscle relaxation. On the other hand, sodium nitroprusside is a functional analog of endothelium-derived relaxing factor and directly activates pulmonary vascular smooth muscle guanylate cyclase to produce cGMP independently of the endothelium.[13]

In this study, dysfunction of cGMP- as well as cAMP-mediated pathways of pulmonary vasorelaxation were exaggerated by the use of bypass. If the mechanisms of pulmonary vasorelaxation are dysfunctional, the balance of pulmonary vascular tone may be shifted toward a net constriction. In addition, dysfunction of the mechanisms of relaxation allow for an exaggerated response to vasoconstricting agents such as hypoxia.[15, 16] Therefore, impaired pulmonary vasorelaxation may result in increased PVR.

Cardiopulmonary bypass is known to activate complement[17] and neutrophils[18, 19] and to increase circulating levels of cytokines such as endotoxin,[19, 20] interleukins,[21, 22] and tumor necrosis factor.[20] When cardiopulmonary bypass is used for lung transplantation, not only is the pulmonary vascular bed subjected to protracted cold ischemia, but the reperfusate may reasonably be expected to produce greater reperfusion injury than when bypass is not used. This may explain the significantly greater pulmonary vasomotor dysfunction produced with the use of bypass in the present study. Further, circulating levels of vasoconstricting substances are increased with bypass.[22] If mechanisms of pulmonary vasorelaxation are impaired in the transplanted lung, these agents may have even greater vasoconstricting effects.

In summary, pulmonary vasomotor dysfunction was greatly exaggerated when cardiopulmonary bypass was used to perform lung transplantation. Impairment of both endothelium-dependent and -independent cGMP-mediated mechanisms as well

as β-adrenergic cAMP-mediated pulmonary vasorelaxation was significantly greater than when cardiopulmonary bypass was not used. These findings suggest that a greater rise in PVR in the transplanted lung may occur if cardiopulmonary bypass is used to perform the procedure.

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Discussion

Dr. Glenn J. R. Whitman *(Philadelphia, Pa.).* What happens to the contralateral lung that has not undergone preservation? What kind of vasomotor dysfunction, if any, does that lung have? More simply phrased, what is the effect of cardiopulmonary bypass on lungs after any heart procedure?

Dr. Fullerton. In the contralateral lung there is no dysfunction of any of the mechanisms of pulmonary vasomotor control as studied in this model. A variety of circulating vasoconstricting agents are present, but without inducing an ischemic injury in the lung, followed by a reperfusion injury, we fail to find any dysfunction of these mechanisms in the contralateral lung.

Dr. Robert A. Guyton *(Atlanta, Ga.).* I have a couple of questions about the mechanisms of cardiopulmonary bypass. First, was any donor blood used in the cardiopulmonary bypass circuit? I presume this was hemodilution without exogenous protein administration. Second, in most of our dog models we give steroids and antihistamine (Benadryl) to reduce the inflammatory response. What

were the conditions of your cardiopulmonary bypass setup?

Dr. Fullerton. We did not use exogenous blood in the animals. The starting hematocrit values were between 42% and 48% and were lowered by hemodilution to the mid-20s. In one dog the hematocrit value was actually lowered to 18%.

We used a bubble oxygenator with a pediatric setup and bicaval cannulation, of course, to allow total bypass. We did not pretreat the animals with anything.

Dr. Anthony L. Moulton *(Providence, R.I.).* Do you think there are any implications from this study about the multiple organ donor (including lung donation) in whom cardiopulmonary bypass and deep hypothermia are used?

Dr. Fullerton. I have not thought about that. If I were to speculate, I do not think there would be much difference in the vasoreactivity of the arteries if deep hypothermia was used to harvest the organs. I think that would protract the cold ischemic injury that we provide clinically, but the injury probably would be comparable.

In another study that we have performed, both in pulmonary arteries and in coronary arteries, we found that this dysfunction is produced on reperfusion of the vascular bed. Dr. Wechsler's group likewise has shown that little vasomotor dysfunction is produced in models of protracted cold ischemia. Thus, assuming that in the donor that you described the deep hypothermia merely provided a longer period of cold ischemia, I do not think there would be much difference from what we have seen here.

Dr. John E. Mayer *(Boston, Mass.).* I suppose the other way of interpreting this is that the vessels that you have are more highly sensitive to the preconstricting phenylephrine that you gave. I presume that you preconstricted all of the vessels to a given tension, or you set the tension, but you must also have some idea about the dimension to which the vessel is prestretched, as well. Do you think either of those two mechanisms is operative?

Dr. Fullerton. You have pointed out an important technical aspect in using this technique. It is important to preconstrict rings of any vascular bed to approximately the same level of tension. In this study all the rings were preconstricted to between 350 and 500 mg of active tension in response to phenylephrine.

As it turns out, the dose-response curve to phenylephrine in the group undergoing cardiopulmonary bypass is shifted a bit to the left, indicating a greater response to a vasoconstricting agent. We think this response is associated with or perhaps even related to a dysfunction in the mechanisms of relaxation. However, for the purposes of the study, the rings are constricted to the same levels of tension in each group, which allows the data to be presented as a percentage.

We examined vessels that are about 1 mm in diameter, the upper limit being about 1.2 mm. For this technique, that is about the smallest vessel that we can study with this apparatus. We were not studying the microvascular bed, which is significantly more difficult to do successfully in the lung than in other organ beds. However, the results in this caliber of vessel are very similar to results of infusion of these agents into the intact animal. We therefore believe the data represent what happens at the microvascular level.

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